

Claims

- [c1] 1. A method for early identification of germline transformed plants comprising the steps of:
- (a) transforming meristematic or cotyledonary tissue with a plant expressible construct comprising at least one nucleic acid sequence encoding a protein to obtain transformed plant tissue;
 - (b) producing a shoot from the transformed plant tissue;
 - (c) growing roots from the shoot;
 - (d) assaying the roots for the presence of the nucleic acid sequence; and
 - (e) identifying roots that assay positive for said at least one nucleic acid sequence as putative germline transformed plants.
- [c2] 2. The method of claim 1 wherein said plant expressible construct comprises a first nucleic acid sequence encoding a protein conferring a trait to said plant, said trait selected from the group consisting of genes that encode for insect tolerance, pest tolerance, herbicide resistance, quality enhancement, yield enhancement, stress tolerance, and environmental tolerance.
- [c3] 3. The method of claim 1 wherein said plant expressible construct comprises at least two nucleic acid sequences each encoding a protein.
- [c4] 4. The method of claim 3 wherein a first nucleic acid sequence encodes a protein conferring a trait to said plant and the second nucleic acid sequence encodes a protein permitting transformed plants to be selected in the presence of a corresponding selection agent.
- [c5] 5. The method of claim 4 wherein the roots are grown in the presence of a selection agent that corresponds to the protein.
- [c6] 6. The method of claim 5 wherein the selection agent is kanamycin.
- [c7] 7. The method of claim 5 wherein the selection agent is glyphosate.
- [c8] 8. The method of claim 1 wherein the transformation is via *Agrobacterium* – mediated or particle–mediated methods.

- [c9] 9. The method of claim 1 wherein the assay is an assay for a protein.
- [c10] 10. The method of claim 9, wherein the assay is selected from the group consisting of ELISA, colorimetric methods, fluorometric methods, and enzymatic methods.
- [c11] 11. The method of claim 1 wherein the assay is an assay for a nucleic acid.
- [c12] 12. The method of claim 11 wherein the assay is selected from the group consisting of PCR, RT-PCR and Southern blots.
- [c13] 13. The method of claim 1 wherein the plant is a dicotyledonous plant.
- [c14] 14. The method of claim 13 wherein the plant is a soybean plant.
- [c15] 15. The method of claim 13 wherein the plant is a cotton plant.
- [c16] 16. A method of obtaining germline transformed plant cells via kanamycin selection comprising the steps of:
 - (a) providing a heterologous DNA construct comprising a promoter functional in plants operably connected to a DNA coding sequence, the coding sequence encoding a protein capable of conferring kanamycin resistance to a plant cell in which the sequence is expressed, and a 3' termination sequence;
 - (b) inserting the DNA construct into the meristematic tissue of a plant embryo;
 - (c) inducing shoot formation from the treated meristematic tissue by culturing the treated meristematic tissue on a culture medium;
 - (d) culturing the shoots on a suitable shooting medium containing kanamycin at a concentration sufficient to significantly inhibit the growth of untransformed plant cells to generate kanamycin resistant shoots;
 - (e) regenerating the shoots of step (d) into genetically transformed plants having increased tolerance to kanamycin relative to wild type plants;
 - (f) assaying the roots from the plants of step (e) for the presence of the DNA construct; and
 - (g) identifying roots that assay positive for the DNA construct as putative germline transformed plants.
- [c17] 17. The method of claim 16, wherein the DNA construct additionally comprises

a gene of interest.

[c18] 18. The method of claim 16, wherein the DNA construct comprises the nptII gene.

[c19] 19. The method of claim 16, wherein the DNA construct is inserted via *Agrobacterium* -mediated or particle-mediated methods.

[c20] 20. A method for early identification of germline transformed plants comprising the steps of:

- (a) transforming meristematic or cotyledonary tissue with a plant expressible construct comprising at least one nucleic acid sequence encoding an antisense gene capable of affecting gene expression of an endogenous gene to obtain transformed plant tissue;
- (b) producing a shoot from the transformed plant tissue;
- (c) growing roots from the shoot;
- (d) assaying the roots for the presence of the nucleic acid sequence; and
- (e) identifying roots that assay positive for said at least one nucleic acid sequence as putative germline transformed plants.

[c21] 21. The method of claim 20 wherein said plant expressible construct comprises at least two nucleic acid sequences, the first encodes an antisense gene and the second encodes a protein permitting transformed plants to be selected in the presence of a corresponding selection agent.

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